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Influence of Light and Darkness on the Pineal Body in *Astyanax mexicanus* (Filippi)

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(Plates I & II; Text-figures 1-4)

INTRODUCTION

THE problem of light sensitivity of the pineal complex in lower vertebrates has been discussed many times. Reactions to light have been demonstrated in both the pineal and the parapineal organ in cyclostomes (Young, 1935; Knowles, 1939) and in the parapineal organ in reptiles (Novikoff, 1910).

In teleosts von Frisch (1911.1, 1911.2) showed that illumination of the pineal region in blinded *Phoxinus laevis* resulted in melanophore reactions. When these reactions were still present after pinealectomy he concluded that light sensitivity is not confined to the pineal body alone but is characteristic of the entire pineal region. E. Scharrer (1928) came to the same conclusion when conditioned reflexes, which in blinded fish were elicited by light stimuli directed toward the region of the head immediately above the pineal, continued to exist after the pineal was destroyed.

Recently the problem was re-examined from new points of view by Breder & Rasquin (1950). These authors observed that phototropism was distinctly related to the degree of transparency of the tissues covering the pineal area. A predominantly positive phototaxis was seen in fishes with exposed pineal areas and a predominantly negative phototaxis in the groups of fishes in which this area is permanently and densely covered. Some phototactic instability was seen in an intermediate group in which the access of light to the pineal is controlled by melanophores. The influence of the pineal area on this behavior was also studied experimentally by covering the heads of blinded fish with India ink which caused the degree of positive phototaxis to be diminished or reversed.

From the results of these experiments and observations, the fact of light sensitivity of the pineal area in teleosts must be considered to be well established. The question remains to what degree the pineal body proper participates in bringing about the phenomena described above. The present study deals with this question by investigating morphologically demonstrable reactions of the pineal body in fish under the conditions of light and darkness.

ACKNOWLEDGEMENTS

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Miss Priscilla Rasquin kindly made available the slides from which the measurements given in Tables 1 & 2, and the photo-micrographs on Plate I, were taken.

Sincere thanks are extended to Dr. George Berg, Department of Biology at the Brookhaven National Laboratories, who most obligingly has given extensive and highly valuable information on the method of decalcification which meets with the requirements of glycogen preservation.

MATERIAL AND METHODS

All experiments and observations were done on the characin *Astyanax mexicanus* (Filippi).

Histological survey and comparative measurements were taken from 38 slides made in connection with studies by Rasquin & Rosenbloom (1954) on the effect of darkness on a number of organs in *Astyanax mexicanus* (Filippi). They contain the pineals of both eyed and blinded fish, part of which had been living

under the normal change of daylight and night, part under darkness. Some of the slides were stained with hematoxyline-eosine, some were unstained. The latter, for the purpose of the present study, were stained with Masson's trichrome stain as described by Rasquin & Rosenbloom (*l.c.*, p. 371). The areas of the pineals were measured by tracing the outlines of cross-sections of the pineal bodies in these slides with a camera lucida at table height; oc. Leitz, 6 × B, obj. 3, 10:1. Every second section was drawn. The areas of each drawing were determined with a planimeter, and the measurements are given in cm^2. For each pineal the average of the areas was taken. These are given in Tables 1 & 2, column 3. The maximum values for each case are given in column 4.

The formation of glycogen in the pineal of *Astyanax mexicanus* (Filippi) under the conditions of light and darkness was examined. Fifty fish, 7½ months old, were used; 25 of them were kept in darkness, 25 in constant light.

Darkness was obtained by keeping the fish in 15-gallon tanks which were surrounded by light-tight wooden boxes and provided with aerating equipment and a device which allowed feeding without exposing the fish to light; the tanks were placed in a darkroom, and red light was used each time it was necessary to lift the cover of the boxes in order to take out the specimens. Conditions of constant light were obtained by placing a fluorescent bulb (General Electric, warm-white, 20 Watt standard, 1860 Lumen) at a distance of 12 inches above the upper edge of the tanks; the brightness at the level of the water was 170 foot candles at the start of the experiment. To insure continual exposure of the fish to light, no plants were introduced into the tanks. Best's carmine stain for glycogen was used for the comparative study of the pineals of the fish that had lived under these conditions for various lengths of time. Controls were made by means of the saliva test. Decalcification was foregone in most of the individuals examined in order to avoid as much as possible any loss of glycogen. Therefore the pineals were taken out of the skulls either before or after fixation. Paraffine sections 5 μ thick were prepared. When, in the course of the investigation, it appeared desirable to examine the distribution of glycogen in the pineal *in situ*, the following method of decalcification proved to be applicable without any apparent detrimental effect on glycogen preservation: the fish were fixed in an ice-cold mixture of 8.5 parts dioxan saturated with picric acid, 1 part formol, 0.5 part glacial acetic acid. This mixture served as a fixer and simultaneously as a decalcifier; decalcification appeared complete after ten days; the specimens were kept cold during the time of decalcification.

Results

In cross-section, the pineal body in *Astyanax* is seen as an oval-shaped organ, the main part of which is located dorsally to the caudal part of the forebrain and immediately beneath the transparent foramen of the skull. It consists of a number of convoluted strands of epithelioid cells surrounding more or less narrow lumina (Plate I, Figure 1). The whole organ is sheathed by richly vascularized connective tissue which also fills the spaces between the epithelial folds. Plate I, Figures 2 & 3, show the pineal as maintained under conditions of light and darkness respectively. Plate I, Figure 1, represents the pineal of a fish which had been living under ordinary laboratory conditions, *i. e.*, the usual daily rhythm of light and darkness in one of the laboratory tanks. Plate I, Figure 2, shows the pineal of a specimen that had been kept for eight weeks in total darkness. Plate I, Figure 3, is taken from a blinded fish which had been living in the dark for 12 weeks. Comparison of these three pictures shows that they differ markedly with respect to the width of the lumen. This is seen to be very narrow in Plate I, Figure 1, *i. e.*, in the fish which had been living under normal conditions. The lumen is wider in the case of Plate I, Figure 2; the folds in the cases of Plate I, Figures 2 & 3, can be seen to be quite flat, surrounding a wide, hardly constricted lumen. Plate I, Figures 2 & 3, also show the pineal to have adopted a less slender, elongated shape than the one seen in Plate I, Figure 1. This is indicative of a tendency towards a change of shape of the pineal frequently seen to occur in the fish kept in constant darkness, where, in extreme cases, the pineal is nearly to the point of becoming a short, obtuse cone with its tip pointing towards the brain.

The picture of the pineal body in fish which had been exposed to constant light is similar to the one seen under the conditions of darkness insofar as the lumina were never seen to be as small and narrowed by convolutions as they are under the normal conditions of alternating day and night. They are, however, less wide than those seen in the more advanced cases in the fish exposed to constant darkness. The tendency to adopt a roundish, plump shape seen in the pineals of fish kept in darkness was not observed in the case of fish which had been exposed to constant light for up to seven months.

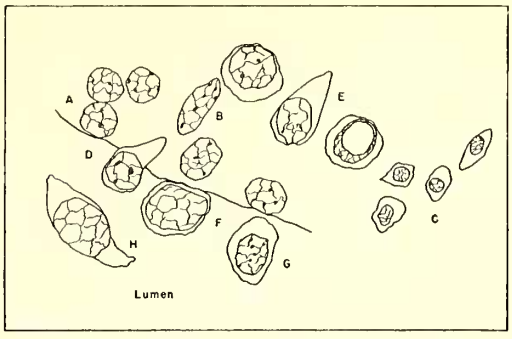
Text-figure 1 shows types of cells ocurring in the pineal body of *Astyanax*. The type most frequently seen is characterized by a rather large, round, vesicular nucleus which, in Mas-

son's trichrome stain, shows a ponceau red-stained chromatic network with one or several small red nucleoli (A, Text-figure 1). The plasma border surrounding these nuclei is very small and, if visible at all, shows a grayish-green color. Besides these cells, which are arranged in an epithelium of two or three layers surrounding the lumen, a number of oval, more or less elongated, nuclei can be seen; in B, Text-figure 1, they appear mostly in a deeper layer of the epithelium, less close to the lumen than the round nuclei (cf. also Plate I, Figures 2 & 3). Only occasionally they are seen protruding towards the lumen and lying between the large round nuclei. Also, irregularly shaped nuclei can be seen. Occasionally there appear nuclei which give the impression of being vacuolized—showing one single, large, completely colorless "vacuole" which is surrounded by deeply stained chromatic material (E, Text-figure 1).

A third group of cells, fewer in number, shows considerably smaller nuclei which are more deeply stained than those in the two groups described above. The narrow plasma border, as far as it is visible, is also more deeply stained. Small processes can be seen in many of them (C, Text-figure 1). Whether or not these processes are nervous in nature cannot be decided with the staining used in this study. The cells belonging to this group seem to be located mainly in the peripheral layers of the pineal body. Frequently they are situated at the dorsal edge. D, Text-figure 1, is typical of the pineal in *Astyanax, i.e.,* a cell of the type A which is protruding into the lumen. Its nucleus is located proximally to the lumen. Its cell body is seen to have formed a process which is extended away from the lumen. Cells of the same type are also frequently seen in the deeper layers of the epithelium, where their processes are extended towards the well-vascularized connective tissue which surrounds the pineal and fills the spaces between the epithelial folds (E, Text-figure 1; see also Plate I, Figures 2 & 3).

The cells, which protrude into the lumen, evidently represent the first stage of a process of migration out of the epithelium into the lumen. Some are partly and others completely detached from the epithelium and are lying freely within the lumen (D, F, G, H, Text-figure 1; see also Plate I, Figures 2 & 3).

Masson-stained slides show the lumen filled with cells, in various stages of disintegration, which have migrated out of the epithelium into the lumen: cells which still show the same structure and staining as the intact cells forming the epithelium, smaller cells with more deeply stained pycnotic chromatin (G, Text-figure 1), and, occasionally, also very large, quasi-



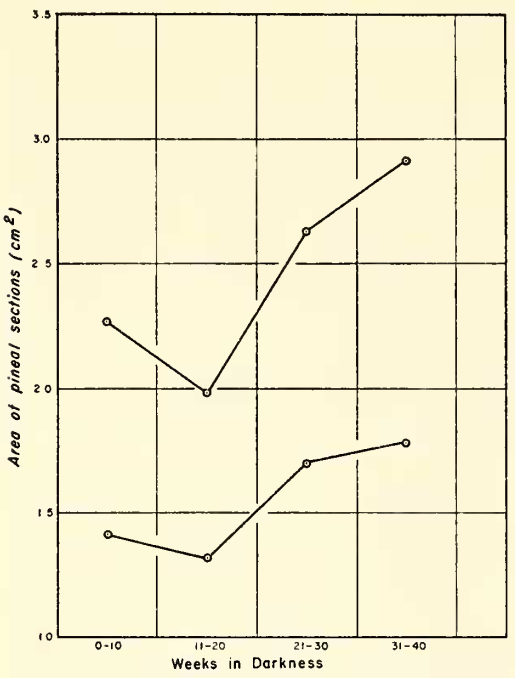
TEXT-FIG. 1. Types of cells occurring in the pineal body of *Astyanax mexicanus*. See text for full explanation.

"inflated" cells with clearly visible grayish-green stained cell body and a faintly ponceau red-stained, enlarged nucleus (H, Text-figure 1). The main part of the lumen is filled with strands of pale fast-green-stained material, which in all probability represents the end product of the disintegrated cells and nuclei. Their outlines are often still recognizable within the fibrous network in the lumen. These contents of the lumen show well in Plate I, Figures 2 & 3, but can also be recognized in the narrow lumen in Plate I, Figure 1. Normally, the cells of the epithelium are tightly packed, forming a well-defined border (Plate I, Figure 1). Under the conditions of prolonged darkness (Plate I, Figures 2 & 3) the nuclei surrounding the lumen are less densely arranged and sometimes the cells appear to be only loosely connected to each other. The oval shaped, elongated nuclei in these latter pineals are more conspicuous than those in the "normal" ones; they are seen to occur most numerously in the vascularized region of the ventral median connective tissue septum (cf. also Plate I, Figures 2 & 3).

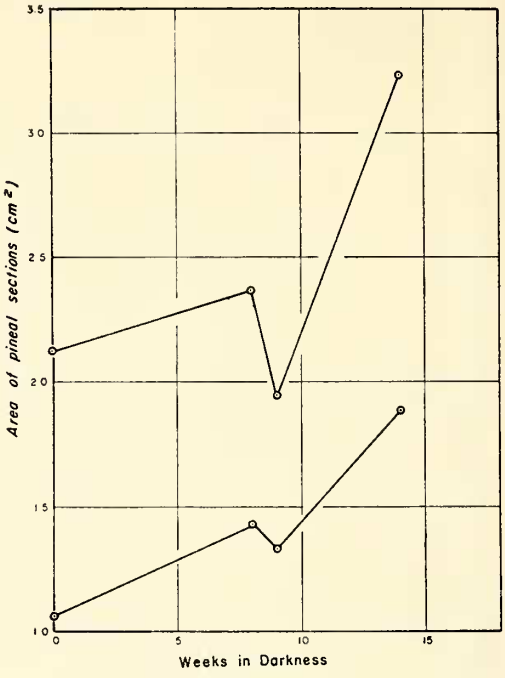
The histological picture of the pineal kept under the condition of constant light resembles the one seen in fish exposed to darkness. The epithelium surrounding the lumen consists of a comparatively small number of round nuclei. Elongated oval nuclei are mostly seen in the deeper layers of the epithelial folds. The small dark-stained cells appear to be numerous, especially at the dorsal edge of the pineal.

The blood supply of the pineal under the various conditions of illumination shows marked differences. Blood corpuscles are abundant in the pineals of the fish raised under normal conditions and under constant light. They are scarce under conditions of darkness.

Considerable amounts of fat have been seen to accumulate in the pineal region in the fish



TEXT-FIG. 2. Graphic representation of the data given in Table 1 (Effect of darkness on the size of the pineal body in eyed *Astyanax mexicanus*). Mean and maximum values.



TEXT-FIG. 3. Graphic representation of the data given in Table 2. These have been calculated by groups, comprising the data obtained in fish which had been living in darkness for less than 10, 20, 30, 40 weeks respectively. Mean and maximum values.

which had been kept in prolonged darkness. Droplets of fat are seen with the dissecting microscope within the loose network of connective tissue which fills the space between the skull and the brain and which surrounds the pineal body. Possibly due to these fat accumulations, the pineal is often seen, in slides, to be located less closely beneath the surface of the skull than in the fish kept under ordinary conditions. This looser connection to the skull could also be noticed when, under the dissecting microscope, the pineal had to be taken out for the purpose of glycogen staining; in general the pineals of the fish kept in darkness were much more easily loosened from their base than those of the fish kept under constant light conditions in which fat deposits at the site of the pineal were not noticed. Breder & Rasquin's (1947) observation of the development of "adipose tissue in great quantities between the bone and the epidermis and between the bone and the meninges governing the brain" but not "at the site of the pineal body" in blind cave fish should bear on this phenomenon. It should be also kept in mind, in this connection, that—according to Rasquin & Rosenbloom (1954, p. 419)—"the dark-reared fish were marked by large accumulations of adipose tissue and wasted musculature, indicating failing adrenal cortical function . . ." This shows that the development of fat in the pineal region of the dark-reared fish can be considered to be part of the phenomenon of the influence of darkness on the fat metabolism in fish.

Tables 1 & 2 and Text-figures 3 & 4 show the results of measurements of the pineals developed under normal conditions as compared with those under darkness. The measurements were made as previously described. Both tables as well as the graphs derived therefrom (Text-figures 3 & 4) show on the whole larger pineals in the fish kept in darkness than in those kept under normal conditions of alternating day and night illumination. The increase in size is not always in proportion to the number of weeks during which the fish were kept in darkness (cf. Nos. 131, 133, 135). A general tendency, however, towards an increase in size of the organ in accordance with the time spent in darkness is evident. Among the 16 cases of fish kept in darkness in Table 2 there are three in which the pineals appear oddly small. One of these cases (No. 150) which, after 15 weeks in darkness showed the extremely small surface average of 0.87 cm^2, is mentioned by Rasquin & Rosenbloom (1954, p. 388) as showing abnormalities

Table 1.[1] Effect of Darkness on the Size of the Pineal Body in Normal Fish

Fish No.	Time in darkness in weeks	Average area of cross-sections in cm.^{2}	Maximum area of cross-sections in cm.^{2}
126	0	1.03	1.94
127	0	1.07	2.52
128	0	1.08	1.94
129	8	1.23	1.94
130	8	1.31	2.59
131	8	1.76	2.59
132	9	1.34	1.94
133	9	1.32	1.94
135	14	1.88	3.23

[1]All fish recorded in Table 1 and Table 2 (column 1) belong to series 4 (eyed fish) and series 5 (blinded fish) in the experiments done by Rasquin & Rosenbloom (1954, pp. 367 & 369). The amount of time spent in darkness has been derived from their Table 2 (*l.c.*, p. 369). The values of the average and maximum areas of cross-sections of the pineal bodies (columns 3 & 4 in Tables 1 & 2) are obtained as indicated in "Material and Methods." The values indicating "Standard Length" and "Greatest Depth" referring to the body size of the fish (column 5 in Table 2) and the "Somatic Index" the quotient resulting from dividing the standard length by the greatest depth (column 6 in Table 2), are taken from Table 4 in Rasquin & Rosenbloom, 1954, p. 380.

in the cellular setup in the transitional lobe of the pituitary and as having "extremely small glands." In fish No. 141 it is mentioned (*l. c.*, p. 397) that — at the time when the fish was sacrificed — "the pseudo-branch appeared normal," indicating that at that time the impact of darkness was not yet seen in this gland. The fact, pointed out by Rasquin & Rosenbloom (*l.c.*, p. 374) "that darkness does not affect all the animals in the same way," may be emphasized in connection with the evaluation of the effects observed.

Columns 6 and 7 in Table 2 indicate the values of standard length and greatest depth of the whole fish and the somatic index calculated therefrom as given in Table 4 of the study by Rasquin & Rosenbloom (1954, p. 380). The values of the sizes of the pineal and the time spent in darkness can thus be compared to these indices of changes in body size under the condition of darkness. Although, again, a "direct correspondence" cannot be derived from this juxtaposition, it can be seen that, while the size of the pineal tends to increase with the length of time spent in darkness, the somatic index tends to decrease—the smallest values being found in those fish which have spent from 21 to 30 weeks in darkness. This means that the larger pineals tend to be associated with the plump-deep-bodied fish as they have been described to

Table 2. Effect of Darkness on the Size of the Pineal Body in Blinded Fish

Fish No.	Time in darkness in weeks	Average area of cross-sections in cm.^{2}	Maximum area of cross-sections in cm.^{2}	Standard length by greatest depth, in mm. (body size of the fish)	Somatic index
136	0	1.27	2.59	55 × 18	3.06
137	0	1.62	2.26	58 × 19	3.05
138	0	1.68	2.65	42 × 12	3.50
139	0	1.08	1.94	45 × 14	3.21
141	8	1.19	1.94	35 × 11	3.18
147	9	1.47	2.00	34 × 11	3.09
148	9	1.55	2.52	29 × 9	3.22
161	12	1.16	1.94	42 × 13	3.23
162	13	1.33	1.94	—	—
150	15	0.87	1.62	—	—
153	19	1.74	2.48	—	—
163	19	1.52	1.94	46 × 13	3.07
164	20	1.36	2.13	39 × 13	3.00
154	21	1.36	2.00	37 × 13	2.85
156	22	1.55	2.59	35 × 14	2.50
157	23	1.85	3.23	45 × 13	3.46
158	25	2.09	3.23	39 × 15	2.60
151	28	2.01	2.59	42 × 13	3.23
160	30	1.68	2.59	40 × 14	2.86
152	38	1.88	3.23	—	—

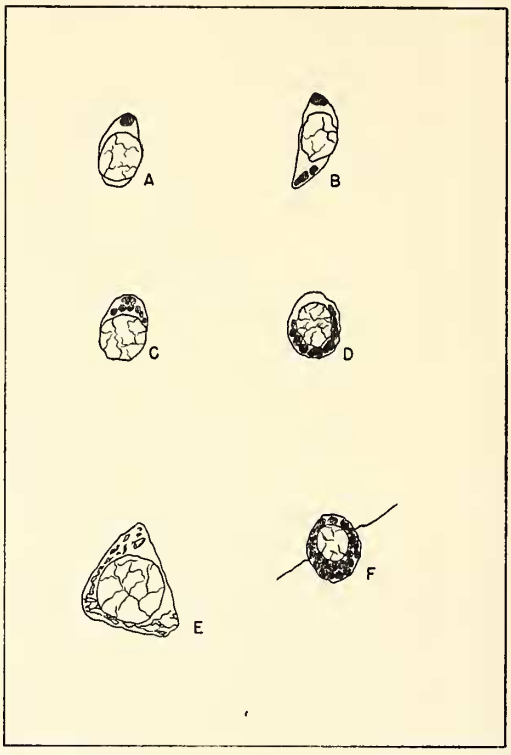
develop in darkness, partly as a result of "rapid accumulation of fat" (Rasquin & Rosenbloom, 1954, p. 372 f.)

The presence of glycogen in the pineal body was shown recently in mammals (Wislocki & Dempsey, 1948; Mikami, 1951). Wislocki & Dempsey (1948) have demonstrated "traces of glycogen" in the parenchymal cells of the pineal in the rhesus monkey; they emphasize that, of all parts of the brain examined by them (neurohypophysis, neurons and neuroglial cells of the white and gray matter in the hypothalamus or in the regions next to the pineal), only the pineal showed the occurrence of glycogen. Mikami (1951), in examining the pineal glands of goats and pigs, found glycogen deposits in all of them and he concludes that some metabolic activity takes place in the pineal which he denotes as being inconsistent with the still widely accepted concept of the pineal being a vestigial, rudimentary organ.

The occurrence of glycogen in the pineal in *Astyanax mexicanus* could be shown by means of the methods described earlier in this study. Plate II, Figures 1, 2 & 3, show pictures of glycogen in the pineal bodies of fish which had been kept under various conditions of illumination. They are taken from slides obtained after fixation and decalcification with the dioxan-mixture described earlier. Plate II, Figure 1, shows the pineal under the "normal" conditions of alternating day and night; Plate II, Figures 2 & 3, are taken from pineals of fish which had been kept for 15 weeks in darkness (Plate II, Figure 2) and in constant light (Plate II, Figure 3) respectively. After staining with Best's carmine, red-stained material, which by means of saliva tests is identified as glycogen, is seen accumulated in the ventral part of the pineals. Bigger and smaller brightly red-stained droplets are suspended within the ventrally located connective tissue septa. Single droplets are seen spreading from there into the more dorsally located parts of the pineal, where they appear within the parenchyma as well as within the lumina. Except for a few small droplets or granules seen in the plasma of some cells, most of the glycogen in these slides is extracellularly located, presenting the phenomenon which is known as "flight of glycogen."

Comparing the three pictures, it is seen that the amount of glycogen under "normal" conditions exceeds considerably the amounts observed under conditions of darkness or of constant light.

A different picture of glycogen occurrence is seen in those slides which were made from isolated pineals. The accumulations of glycogen in



TEXT-FIG. 4. Occurrence of glycogen in cells of the pineal body. See text for full explanation.

the ventral part of the pineal obtained when using the dioxan-method did not appear in these slides. Instead, Best's carmine-stained small granule-like droplets appear both extra- and intracellularly throughout the entire pineal, within the tissue as well as within the lumen. Text-figure 4 shows various pictures of glycogen within the cell. It is most frequently found in the cell with the round vesicular nucleus. There are cells containing just one small red droplet within the cytoplasm (A, Text-figure 4); in others the amount of droplets is increased (B, C, D, Text-figure 4). In other cases the whole cell body is filled with red droplets (F, Text-figure 4). Occasionally the glycogen is seen to fill the cell as a pale pink-stained homogeneous mass. The glycogen-filled cell pictured in F, Text-figure 4, is about to enter the lumen. Glycogen-filled cells within the lumen present the same stages of disintegration which were found in the histological picture of the pineal body after Masson staining (Text-figure 1), as described above. E, Text-figure 4, shows one of the big "inflated" cells within the lumen corresponding to those shown in F, G, H, Text-figure 1. Its glycogen content appears paler and less densely packed than in the intact cells. Red-stained

droplets and irregular fibrous strands are found lying free in the spaces between the disintegrating and disintegrated cells. Occasionally, droplets and strands of red-stained material are also seen in the parenchyma of the pineal body. However, it is difficult to decide whether these result from a process of disintegration of glycogen-bearing cells or from cases of "flight of glycogen." The features described are found in the pineals of fish kept in darkness as well as in those kept in constant light. In all probability, they can be interpreted as the morphological aspect of a process of apocrine secretion by which glycogen is being transported into the lumen where it is released. No marked quantitative differences such as were shown in Plate II, Figures 1, 2 & 3, can be demonstrated in these slides obtained from isolated pineals. It is thus a favorable circumstance that the two methods of demonstrating glycogen in the pineal, as applied in this study, are complementary to each other. The second has the advantage of showing the cytological details of glycogen occurrence within the cell. The advantage of the first method, apart from showing the pineal *in situ*, results from the otherwise undesirable phenomenon of "flight of glycogen" by which it was possible to compare the amounts of glycogen produced under the experimental conditions used. In combining the results of both methods it may be stated that at least part of the secretory processes, which in all probability take place in the pineal body, deals with the formation of glycogen. This is seen to be produced in greater quantities in fish which are kept under normal conditions of alternating day and night than in those which were kept in darkness or in constant light.

Discussion

Morphological and physiological changes in the pineals of *Astyanax mexicanus* have been shown to occur under the influence of light and darkness. The size of the organ increases when the fish are kept in darkness. The lumen is narrow under normal conditions of illumination and gets wider and less constricted by invaginations on exposure to either darkness or constant light. Red blood corpuscles are abundant in the fish kept in alternating light-darkness conditions; they are scarce in the fish kept in darkness. The number of cells surrounding the lumen decreases in both constant darkness and constant light; they are less closely connected to each other than in the fish raised under normal conditions and in extreme cases the epithelial unit seems to disintegrate. The amounts of glycogen produced under the conditions of constant darkness and constant light respectively are smaller than those produced under normal illumination.

The studies by Rasquin (1949) and by Rasquin & Rosenbloom (1954) have provided a great deal of information on the effect of darkness on the endocrine system, on growth, body shape, the kidneys and a number of other organs in *Astyanax mexicanus*. Most of the organs examined were distinctly affected when the fish was kept in darkness, *i. e.* in living conditions to this extent rescmbling those of its cave derivatives. "A condition of hormonal imbalance ordinarily inhibited by the presence of light and marked by somatic and pathological modifications" (Rasquin & Rosenbloom, 1954, p. 419) was seen by them to be produced in the fish raised in darkness—a condition which "can be considered a situation of long continued stress" (*l. c.*, p. 419).

The stimulus of constant light has been used in experiments on mammals, reptiles and amphibians. Prolonged application of constant illumination was seen to result in morphological and physiological changes, indicative of dccreased function, in the pituitary (Stutinski, 1936; Florentin & Stutinski, 1936; Fiske, 1941; Woitkewitsch, 1944.1); in gonads (Fiske, 1941; Pomerat, 1942); and in the thyroid (Woitkewitsch, 1944.2, 1946; Puntriano & Meites, 1946).

According to Bissonnette (1938) it can be considered establishcd that "effects of light and darkness" are "mediated by the eyes, optic nerve and pituitary and accompanied by both cytological and physiological changcs in the gland and in pituitary activity" (*l. c.*, p. *372*). The pituitary, thus, is the mediating agent through which the effect of illumination is transferred to its target-organs. With reference to this fact it is interesting to note that Rasquin & Rosenbloom (1954, p. 306), among all the organs examined, found "no effects" (*sc.* "of darkness") in the pancreatic islets and corpuscles of Stannius, neither of which "has yet been proved to be directly under stimulation by the pituitary."

The pineal body also has never yet been found to be under direct stimulation by the pituitary. However, in contrast to the pancreatic islets and Stannius' corpuscles, the pineal in *Astyanax mexicanus* does show morphological and physiological changes under the influence of darkness and constant light. These changes, therefore, are strongly suggestive of being produced by direct photic stimulation of the pineal itself.

This conclusion may be considered to be supported by the fact that an independent reaction of the pineal body towards photic stimuli is

consistent with our knowledge about sensory cells in the pineal of some teleosts.

"Sensory cells" in the pineal in teleosts have been described by Studnicka (1905), Holmgren (1920) and Friedrich-Freksa (1932). Holmgren, in *Osmerus eperlanus* (1920), describes a very specific kind of cells which he considers to be photoreceptory, similar to those which he had described earlier in *Squalus acanthias* and in *Rana* (1917/18). In these he distinguishes two kinds of processes ("Aussen" and "Innenglied") which are seen to show special structures ("Spitzenstueck" and "Spiralfaden"). These processes, according to this author, detach themselves from the cells and migrate into the lumen where they are dissolved. Similar pictures are described in *Dermogenys pusillus* by Friedrich-Freksa (1932), who considers these cells to be sensitive to blood pressure fluctuations, and he describes a complicated system of capillaries, part of which he assumes to be filled with a blood pressure-regulating secretion produced by the pineal cells.

The elaborate structures described by these investigators could not be found in *Astyanax* with the methods used in this study. There are indications, however, that a sensory apparatus does exist in the pineal of *Astyanax mexicanus:* the small dark-stained cells (C, Text-figure 1) which were seen scattered in the pineal parenchyma are often seen to show processes of various shape; these appear more numerous at the dorsal edge of the pineal body than in other parts of the organ and they stain well with intravital methylene blue staining. Furthermore, by means of this staining, the existence of free nerve endings within the pineal parenchyma can be shown. These studies are still in progress and will be published at a later date. The possibility shoud be kept in mind that *Astyanax mexicanus* is not a particularly favorable object for the study of a sensory apparatus in the teleostean pineal. Other species seem to display better-defined specific structures. Further study in this direction therefore should be extended to other species.

Excluding the controversial question of direct effect of light on the skin, there are three ways in which light stimulates the fish: (a) directly through the eyes, (b) indirectly through the pituitary, (c) directly through the pineal body. With respect to the possible significance of this third way it may be emphasized that there is increasing evidence of an antagonistic relationship between the pineal body and the anterior lobe of the pituitary gland, as recently shown by Thiéblot (1954). The author of the present study has shown, in a previous paper (Grunewald-Lowenstein, 1952), that the activity of the pineal body affects the autonomous nervous system by increasing the activity of its sympathetic part and that, antagonistically, the activity of the anterior lobe of the pituitary increases the activity of the parasympathetic part of the autonomous nervous system. In view of these facts the concept might be advanced that, besides its main access to the body through the eyes, light, in fish, influences the vegetative neurohumoral system of the body by means of both the pituitary gland and the pineal body, the effects of which are antagonistic to each other. This concept should be considered in further research.

Summary

1. The influence of darkness and of constant light on the pineal body of *Astyanax mexicanus* (Filippi) was examined.
2. The influence of darkness shows in increased size of the organ, increased width of the lumen, diminished depth of the epithelial folds and gradual disorganization of the epithelium. Changes of the shape of the organ are observed. Accumulations of fat develop in its surroundings. The number of blood corpuscles is diminished.
3. Exposure of the fish to constant light results in similar pictures: the lumen is wide, the depth of the folds is diminished, the epithelium is less compact than under normal conditions. No effect on the shape of the organ, on the number of blood corpuscles or on the development of fat was observed.
4. Glycogen is seen to develop in the pineal under the conditions of darkness and of constant light as well as under normal conditions. Its amount in darkness and in constant light is less than in alternating light and darkness.
5. The pictures of the occurrence of glycogen in the pineal cells in *Astyanax mexicanus* are indicative of a process of apocrine secretion by means of which glycogen is emptied into the lumen.
6. The morphological and physiological changes in the pineal body in *Astyanax mexicanus* can be considered to be produced by direct photic stimulation of the organ.

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EXPLANATION OF THE PLATES

Plate I

Transverse sections of the pineal body of *Astyanax mexicanus*. Fix. Bouin. Masson's Trichrome Stain. Magnification 340×.

Fig. 1. Fish kept under the normal change of daylight and night. The lumen is extremely narrow. The epithelial folds are deep. The cells in the epithelium surrounding the lumen are closely arranged. Richly vascularized connective tissue fills the spaces between the folds.

Fig. 2. Fish kept in darkness (8 weeks). The lumen is wide. The epithelial folds are less deeply invaginated.

Fig. 3. Blinded fish kept in darkness (30 weeks). The lumen is wide. The cells in the epithelium are less numerous and less closely connected than in Text-figure 1 A and B.

Plate II

Accumulation of glycogen in the ventral part of the pineal body in *Astyanax mexicanus*. Transverse sections. Fix: Dioxan–Picric Acid–Formal–Glacial Acetic Acid. Stain: Best's Carmine. Magnification: 1000×.

Fig. 1. Glycogen content in the pineal body of *Astyanax mexicanus* kept under the normal change of daylight and night.

Fig. 2. Glycogen content in the pineal body of *Astyanax mexicanus* kept in darkness (15 weeks).

Fig. 3. Glycogen content in the pineal body of *Astyanax mexicanus* after 15 weeks in constant light.